# FINAL SYNOPSIS OF Ph.D.THESIS

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## Title of the Thesis

**Physiological response of coconut (*Cocos nucifera* L.) seedlings to elevated $CO_2$ and temperature**

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# Physiological response of coconut (*Cocos nucifera* L.) seedlings to elevated $CO_2$ and temperature

## 1. Introduction

The assessment reports brought by IPCC (AR4 2007) provided scientific evidence to the fact that human activities may already be influencing the climate. United Nations Framework Convention on Climate Change (UNFCCC) has defined climate change as "a change of climate attributed directly or indirectly to human activity which alters the composition of the global atmosphere and that is in addition to natural climate variability observed over comparable time periods". It is projected that the frequency of heat waves, cold waves, frost events, droughts, heavy precipitation events and floods will increase apart from increase in temperature, and change in rainfall. All these significantly influence the plant growth and crop production, particularly of perennial crops. Coconut (*Cocos nucifera* L.) is one of the most useful palms in the world grown in about 86 countries in the tropical belt. In general it thrives in a climate characterized by warm and humid conditions (Menon and Pandalai, 1960). Hence change in climate may significantly influence the growth, development and yield of this plantation crop. Simulation analysis using InfoCrop-COCONUT projected spatial variations for climate change impacts on yield with coconut plantations in western coast region likely to benefit while those in eastern region may be adversely affected (Naresh Kumar, 2008; Naresh Kumar and Aggarwal, 2009). Hence it is essential to understand the physiological responses of coconut to elevated [$CO_2$] and rise in atmospheric temperatures [T] - as climate change impacts on plants are mainly through these two factors apart from variability in rainfall- in order to identify the adaptation strategies to reduce the adverse impacts of climate change and to maximize beneficial influences. Such studies are conducted using controlled environmental facilities viz., Free Atmospheric $CO_2$ enrichment (FACE) or Open Top Chambers (OTC). With a few exceptions (Tognetti *et al*., 2001) most studies targeted response of plants to double the ambient $CO_2$ concentration only (Engloner *et al*., 2003; Eguchi *et al.*, 2004). Since ambient $CO_2$ will elevate gradually, it will be more appropriate to study the effect of at least two levels of $CO_2$ concentrations on plants. Even though ample literature is available on impact of

elevated [$CO_2$] and temperature on annual crops (Batts *et al*., 1998; Perez *et al*., 2007; Young *et al*., 2008), only limited studies are conducted on perennial crops (Dieter Overdick and Karin Fenselau, 2009; Warren *et al*., 2011a) and none on coconut. In view of above mentioned background, this investigation was carried out using OTC facility at CPCRI by growing the coconut seedlings in elevated [$CO_2$] and temperature conditions with objectives to

1. Estimate the morphological growth response of coconut seedlings to elevated [$CO_2$] and temperature
2. Study the impact of increased [$CO_2$] and temperature on anatomical features of coconut seedlings
3. Estimate physiological parameters related to photosynthesis in above seedlings

## 2. Brief review of literature

Plants responded to elevated [$CO_2$] by increasing their shoot height (Saebo and Mortensen, 1996), leaf area and with added number of leaves (Soares *et al.*, 2008), and elongated roots (Day *et al*., 2006). A positive shift in ambient temperature will have significant impact upon plants. Most common response is retarded growth (Olszyk *et al*., 1998). Not only morphological but anatomical responses were also studied. Stomatal density reduced under elevated [$CO_2$] (Engloner *et al.*, 2003) and temperature (Beerling and Chaloner, 1993) while under elevated [$CO_2$] leaf cuticle thickened reducing the transpiration rate (North *et al*., 1995) and improving leaf water potential (Xiao *et al*., 2005). Most plant species had thicker leaves under enriched [$CO_2$] levels (Tepping and David, 1999). On the other hand plants exposed to elevated temperature had thinner leaves (Mei *et al.*, 2007). Some coconut cultivars such as WCT, WCT x COD found to be tolerant to drought and able to acclimatize to drought condition with anatomical modifications (Naresh Kumar *et al*., 2000).

Elevated [$CO_2$] and temperature influenced physiology of plants in several ways. Chlorophyll, which is a major photosynthetic pigment, found to decrease under elevated [$CO_2$] and temperature (Ommem *et al*., 1999; Perez *et al*., 2007; Wang *et al*., 2009). However, dark and light adapted photochemical efficiencies increased under elevated [$CO_2$] (Gutierrez *et al*., 2009). Plants had improved leaf water potential and membrane

stability when exposed to elevated [$CO_2$] (Schwanz and Polle, 2001; Atwell *et al*., 2007). Elevated [$CO_2$] enhanced net photosynthetic rate in plant species due to abundant availability of substrate for carboxylation reaction and also due to reduced oxygenation reaction of RUBISCO enzyme (Long, 1991). Stomatal conductance and transpiration rates were inversely related to increased $CO_2$ concentration, which enabled plants to have better water use efficiency (Young *et al*., 2008; Warren *et al*., 2011b). The $CO_2$ fertilization stimulated photosynthesis and led to higher biomass production (Matsunami *et al*., 2009). On the other hand, elevated temperature either increase (Vu and Allan Jn, 2009) or decrease (Pettigrew, 2008) biomass accumulation, depending on the ambient temperature regimes.

## 3. Methodology

Seedlings of three coconut cultivars viz., WCT, LCT, COD and two hybrids viz., WCT x COD and COD x WCT were grown in six $4m^3$ OTCs at Central Plantation Crops Research Institute, Kasaragod (12˚18' N, 75˚ E, ~7m AMSL). Three months aged seedlings were planted in poly bags and raised with adequate irrigation and uniform nutrition in the form of vermicompost. After six months, well established seedlings were transferred to OTCs. In OTCs, they were exposed separately to i) elevated [$CO_2$] at 550 µmol $mol^{-1}$ in one OTC, ii) elevated [$CO_2$] at 700 µmol $mol^{-1}$ in another OTC, and iii) elevated air temperature at $2^oC$ ([T+2°C]) above chamber control in two OTCs. Apart from these, one set of seedlings was maintained in the fifth and sixth OTCs without any additional treatment to serve as the chamber control (CC). This was done to discount the chamber effects on the growth of seedlings. In each OTC eight seedlings/ cultivar were maintained. Apart from these, one more set of seedlings was also maintained under shade net (SN) - with 75% light transmittance to serve as the absolute control. The $CO_2$ levels were maintained at set level of 550 and 700 µmol $mol^{-1}$; with constant supply of commercial $CO_2$ through automated $CO_2$ supply and monitoring SCADA system attached to data logger and a computer. Rise of temperature by 2˚C above chamber control was realized by hot air-blowing, which was dynamically controlled by the SCADA system. The temperature, relative humidity and $CO_2$ levels were monitored continuously and were logged at 10 minutes interval.

### 3.1. Growth parameters of coconut seedlings to elevated $CO_2$ and temperature

Measurements on morphological traits such as shoot height, collar girth and total number of leaves were made on seedlings at six monthly intervals from the date of transfer of seedlings to OTCs. Total leaf area, specific leaf weight and specific leaf area were calculated by sampling the leaf tissue from middle leaflets of third leaf from top. Root length, number and volume were also measured.

### 3.2. Anatomical features of coconut seedlings

To study anatomical structure, leaf segments (4 cm length), from middle portion of middle leaflets of the third leaf from top were sampled at about 10 AM in a clear sky day. They were immediately fixed in Carnoys fixative (chloroform: alcohol: glacial acetic acid mixture in 30:60:10 ratio) for 48 hours and permanent slides were prepared. Stained sections were observed under light microscope (Leitz Diaplan, Germany) connected with a Leica camera. At least ten digital images were captured for each section in 25x and observations were recorded on several anatomical features using Leica Q win (Leica, Germany) image processing software. To study the structure of stomatal complex, clear transparent nail varnish was applied at about 10 AM on a clear sky day on the abaxial surface of leaf. For each cultivar epidermal impression was taken from six seedlings. Digital images of epidermal impressions were captured under Leitz Diaplan (Germany) microscope, connected with camera; and were analyzed stomatal density and for area of stomatal aperture and guard cells.

### 3.3. Physiological parameters related to photosynthesis

In order to quantify the response of gas exchange characteristics under elevated $CO_2$ and temperature conditions, parameters such as net photosynthetic rate, stomatal conductance, transpiration rate, leaf internal carbon dioxide concentration were measured in seedlings following method standardized for coconut seedlings (Rajgopal *et al*., 2000) using LICOR-6400 (USA) gas analyzer. Chlorophyll fluorescence transients were recorded in coconut leaves using PAM 2000 portable chlorophyll flouormeter (Walz, Germany) equipped with special fiber optics, 2010F following the method described by (Naresh Kumar and Kasturi Bai, 2009).

Apart from these, photosynthetic pigments such as chlorophyll a, chlorophyll b were analyzed following Arnon (1949). Leaf water potential was recorded using SKPM

pressure chamber. The recordings were done in a bright sunny day between 9.30 and 11.30 AM following the standardized method for coconut (Rajagopal *et al*., 1987). Leaf membrane stability in terms of electrolyte leakage (Lu *et al*., 2003) was analyzed. Oven dried and acid digested leaf samples from third leaf from top were used for estimation of sodium and potassium concentrations using flame photometer (Genway PPP7, flame photometer, UK); for estimation of phosphorus using spectrophotometer (UV Visible Spectrophotometer, Shimadzu, Japan); and for estimation of iron, copper, manganese, magnesium, calcium and zinc using atomic absorption spectrum (GBC Avantha, Australia). Nitrogen content in oven dried leaf tissue was estimated using Kjeldal system (Gerhardt vapodest, UK). All data were statistically analyzed using SPSS (v10) software package and critical differences were calculated to compare the means.

**4. Salient findings**

Growth of coconut seedlings in OTCs was comparatively less than those grown in shade net. In order to discount the chamber effects, performance of seedlings exposed to elevated $CO_2$ and temperature was compared to that of seedlings grown in control chamber. The results indicated that coconut seedlings respond to elevated [$CO_2$] and temperature at various levels i.e., morphological, anatomical and physiological levels. The morphological responses in elevated [$CO_2$] included improved seedling height, collar girth (~6 and 15% at 550 and 700 µmol $mol^{-1}$ respectively), specific leaf weight, leaf area (~13 and 17% at 550 and 700 µmol $mol^{-1}$ respectively), root length and root volume. It also led to anatomical changes such as increased thickness in leaflet (~17 and 12% respectively at 550 and 700 µmol $mol^{-1}$) and thickness of mesophyll, epidermis and cuticle (~47 and 26% respectively in 550 and 700 µmol $mol^{-1}$). Among the physiological parameters, elevated [$CO_2$] increased photosynthetic rate (~42 and 75% respectively at 550 and 700 µmol $mol^{-1}$), leaf internal $CO_2$ concentration ($C_i$), $P_N/C_i$ ratio and water use efficiency (~126 and 81% respectively at 550 and 700 µmol $mol^{-1}$), apart from maintaining higher leaf water potential (~14 and 32% respectively at 550 and 700 µmol $mol^{-1}$) and membrane stability. The bio-physical characteristics of chlorophyll fluorescence such as $F_m$, $F_v$, $F_v/F_m$, electron transport rate and photochemical quenching were more in seedlings exposed to elevated [$CO_2$]. Seedlings had higher concentration of nutrients such as calcium, potassium and sodium. Total dry matter (~14 and 57%

respectively at 550 and 700 µmol $mol^{-1}$) and root: shoot ratios (~7%) were more in seedlings exposed to elevated [$CO_2$] treatments.

Exposure to elevated [$CO_2$] caused significant reduction in specific leaf area and number of roots (~6%). Stomatal density (-10 and -14% respectively at 550 and 700 µmol $mol^{-1}$) and aperture size, and guard cells area were also less apart from lower rate of transpiration and stomatal conductance as compared to the chamber control. It also reduced concentrations of chlorophyll (~22%), manganese and copper in leaf.

Seedlings grown in elevated temperature, in general, had slightly higher leaf area (~4%) and specific leaf area. Leaves had thicker cuticle (~24%) and epidermis due to elevated temperature. Elevated temperature did not significantly change shoot height, collar girth, number of leaves, $F_v/F_m$, photosynthetic rate, $C_i$, $P_N/C_i$ and total biomass as compared to that in chamber control. However, seedlings grown in elevated temperature had lengthier roots (30% more than that in chamber control).

On the other hand elevated temperature condition reduced specific leaf weight, thickness of leaflet (~4%) and mesophyll layer (~6%). Leaflets had reduced stomatal density (~6%), index (~13%) and aperture, and also reduced guard cells area. Apart from these, chlorophyll concentration (~11%), electron transport rates, membrane stability and concentration of some of leaf nutrients such as iron, manganese, copper, calcium and phosphorus reduced in elevated temperature conditions.

Even though above mentioned trends were generally observed in coconut seedlings exposed to elevated [$CO_2$] and temperature, responses significantly varied depending on cultivar. For instance, COD x WCT was more responsive in terms of specific leaf weight, leaf area, chlorophyll a/b, thickness of leaflet, thickness of mesophyll, upper cuticle, photosynthetic rate, $g_s$, root volume and concentration of calcium and sodium in leaf in elevated $CO_2$ both at 550 and 700 µmol $CO_2$ $mol^{-1}$. Whereas collar girth, transpiration rate, number of roots, dry matter and ratio of root: shoot dry matter, were more in COD cultivar. On the hand, $F_m$, $F_v$, $F_v/F_m$ and leaf potassium concentration were more in WCT.

Similarly, root length, lower cuticle and epidermis cell thickness, xylem vessel diameter, membrane stability, electron transport rate and leaf nutrients like iron and phosphorus concentrations were more in WCT x COD. While, chlorophyll a, b and total, leaf water potential, $P_N/C_i$, $P_N/g_s$ and xylem vessel lignificantion increased in LCT.

As in case of elevated $CO_2$, elevated temperature also resulted in differential response of coconut cultivars with respect morphological, anatomical and physiological parameters. Elevated temperature resulted in taller seedlings with more collar girth in COD x WCT hybrid as compared to those grown in control OTC and also among cultivars. They also had thicker leaflets, thicker mesophyll layer and cuticle apart from having higher net photosynthetic rate, electron transport rate, transpiration rate, concentrations of leaf nutrients such as copper, potassium, sodium and phosphorus. These seedlings also had more number of roots, root volume, root length and shoot dry matter. However, elevated temperature had significantly reduced stomatal density in this hybrid. Elevated temperature increased xylem vessel lignification, stomatal conductance, root dry matter and ratio of root/shoot dry matter more in COD than in other cultivars. Among the cultivars, WCT responded more in terms of number of green leaves, leaf area, specific leaf weight, upper cuticle thickness, $F_v$, $F_m$, $F_v/F_m$, leaf water potential, membrane stability which increased in elevated temperature conditions. While, concentration of chlorophyll b and total chlorophyll and xylem vessel diameter increased more in WCT x COD. Whereas, LCT responded more in terms of increase in guard cells area, concentration of chlorophyll a, a/b ratio, $P_N/C_i$ , $P_N/g_s$ and concentration of leaf nutrients such as calcium, iron, magnesium and manganese.

In spite of noted differences in parameter dependent response of a cultivar and minor changes in trends at 550 and 700 µmol $mol^{-1}$ concentrations of $CO_2$, overall results indicate COD x WCT and COD as most responsive to elevated $CO_2$ conditions. Whereas, in elevated temperature conditions, COD x WCT and WCT were more responsive.

## 5. Discussion

As the micro-climate in open top chambers (OTC) is different from that in shade net, growth of seedlings in OTCs were comparatively lesser than those grown in shadenet.

Therefore, response of seedlings grown in elevated [$CO_2$] and temperature were compared with those grown in control chamber, to avoid chamber effects. Results indicate that coconut seedlings respond to elevated [$CO_2$] and temperature by modifying the anatomical parameters such as reduced number of stomata, smaller stomatal aperture, modification of stomatal complex and deposition of epiculticular wax. These modifications helped seedlings to maintain the leaf water potential.

The elevated [$CO_2$] led to increased photosynthetic rates and thereby helping plant to accumulate more biomass, even though increase in leaf area was not significant, leading to increased specific leaf weight. Availability of additional biomass seems to have helped for development of thicker and lengthier roots resulting in larger root volume. Improved photosynthetic accumulation and improved mining of water and nutrients from soil helped seedlings to accumulate more biomass in elevated [$CO_2$] conditions as exhibited by taller and vigorous seedlings as compared to those in control chamber.

Increase in specific leaf weight was coupled with anatomical changes such as thicker cuticle, epidermis and mesophyll layer. Results indicate that elevated [$CO_2$] did not enhance the chlorophyll synthesis, but due to increased specific leaf weight the concentration per unit weight decreased. Despite of reduction in concentration of chlorophyll, $P_N$ rate increased in elevated [$CO_2$] indicating adequate amount of chlorophyll well spread in thicker mesophyll layer leading to increased mesophyll carboxylation efficiency. Chlorophyll maturity is also increased in elevated [$CO_2$] as indicated by higher $F_v/F_m$ ratio and $F_m$ value.

On the other hand in elevated temperature, smaller stomatal aperture reduced stomatal conductance leading to higher water use efficiency. In spite of having photosynthetic rates and photochemical efficiencies similar to those in chamber control, elevated temperature reduced leaflet thickness and increased specific leaf area indicating higher maintenance respiration. Coconut seedlings when grown in elevated temperature conditions had fewer but lengthy roots instead of initiating more roots.

The cultivars were ranked based on their morphological, anatomical and physiological responses to elevated [$CO_2$] and temperature. Since, the magnitude of response varied among cultivars for individual parameters, overall response was considered in raking most responsive cultivar. From this analysis COD x WCT was found to be most responsive to elevated [$CO_2$] and temperature under water non-limiting conditions among cultivars/hybrids chosen for this study.

## 6. Summary and conclusions

In general, elevated [$CO_2$] benefited the growth and development of coconut seedlings as they assimilated more carbon dioxide, due to higher photosynthetic rates and larger leaf area, resulting in significant increase in shoot and root biomass. The leaf water balance was maintained by thicker cuticle, by reduced stomatal density and by regulation of stomatal conductance in both elevated [$CO_2$] and temperature. As seedlings maintained similar photosynthesis and dry matter in spite of slightly lower leaf water potentials in elevated temperature (+2$^oC$) conditions as compared to those in chamber control, it may be concluded that rise in temperature up to 2°C above 31°C (the annual mean maximum temperature of experimental site) may not be deleterious to coconut, provided water is non-limiting. Therefore it may be concluded that future climate change may improve growth and biomass of coconut seedlings making them more vigorous.

## 7. Publications

**Muralikrishna, K. S**., Naresh Kumar, S. and John Sunoj, V. S. 2009. Elevated $CO_2$ and temperature reduce Stomata density in coconut. Proceedings of national workshop on climate and development at Kerala Agricultural University, Thiruvananthapuram, PP: 153-158.

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John Sunoj, V. S., Naresh Kumar, S. and **Muralikrishna, K. S.** 2010. Variation in total phenol concentration in coconut seedlings under elevated $CO_2$ and temperature conditions. International conference on climate change held during 24$^{th}$ to 26$^{th}$ October at Cochin (accepted for publication in Indian Journal of Marine Science special edition).

**Muralikrishna, K. S**., Naresh Kumar, S. and John Sunoj, V. S. 2010. Elevated $CO_2$ and temperature affect leaf anatomical characteristics in coconut. International workshop on climate change held during 28$^{th}$ to 31$^{st}$ October at Lakshadweep (accepted for publication in Indian Journal of Marine Science special edition).

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